Obtenção dos tempos de equilíbrio e coeficientes de difusão de ácido e de sal para desenhar o processo de marinado de filés de *Engraulis anchoita*

Obtainment of equilibrium times and diffusion coefficients of acid and salt to design the marinating process of Engraulis anchoita fillets

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Abstract

Simultaneous diffusion of sodium chloride and acetic acid in anchovy fillets was studied. The results show that equilibrium conditions between the fillets and the marinating solution were reached according to the K values obtained close to unity. The marinating solution was stirred to prevent the formation of a diluted surface layer on the fish fillets. The equilibrium times for acid and salt were higher for the samples stirred during marinating. The sodium chloride and acetic acid uptake during marinating of anchovy fillets can be explained by Fick's law. The values of the diffusion coefficient for acetic acid at 20 °C were 3.39×10^{-6} and 3.49×10^{-6} cm^2/seconds for marinating with and without agitation, respectively. The value of the diffusion coefficient for sodium chloride for marinating without agitation was 2.39×10^{-7} cm^2/seconds, while it could not be obtained for marinating with agitation.
Keywords: *anchovy; marinating; diffusional analysis.*

Resumo

Foi estudada a difusão simultânea de cloreto de sódio e ácido acético em filés de anchoíta. Os resultados mostram que foram alcançadas condições de equilíbrio entre os filés e a solução de marinado, de acordo com os valores K obtidos para fechar a unidade. A solução de marinado precisou de agitação para evitar a formação de uma camada superficial diluída nos filés de pescado. Os tempos de equilíbrio para ácido e sal foram maiores para as amostras agitadas durante o marinado. O gasto de cloreto de sódio e ácido acético durante o marinado dos filés de anchoíta pode ser explicado pela lei de Fick. Os valores do coeficiente de difusão para ácido acético a 20 °C foram $3,39 \times 10^{-6}$ e $3,49 \times 10^{-6}$ cm^2/segundos para marinado com e sem agitação, respectivamente. O valor do coeficiente de difusão de cloreto de sódio para marinado sem agitação foi de $2,39 \times 10^{-7}$ cm^2/segundos, contudo, para marinado com agitação, não pôde ser fixado.
Palavras-chave: *anchoíta; marinado; difusão análise.*

1 Introduction

When marinating foods, pieces of fresh or defrosted food are immersed in solutions of low pH and high salt content to obtain a product with pH lower than 4.5 and with the desired sensorial characteristics (MC LAY, 1972). Differences of acid and salt contents in food and marinating solutions cause simultaneous diffusion of acid and salt in the food and water out of the food. The study of diffusional changes in each food and the determination of equilibrium times are very important and necessary to determine marinating conditions.

Telis et al. (2004) determined the apparent diffusion coefficient for sucrose, NaCl and water during osmotic dehydration of tomatoes. Turhana and Kaletunc (1992) determined a modeling of salt diffusion for their work on white cheese during long-term brining, Pajonk et al. (2003) conducted an experimental study and modeling of NaCl diffusion coefficients during hard-cheese brining and Gerla and Rubiolo (2003) studied the simultaneous diffusion of NaCl, lactic acid and water in cheese during brining. Deng (1977), Favetto et al. (1981), Zugarramurdi and Lupin (1980) and Graiver et al. (2006) determined some kinetic data on the penetration of salt in meat and fish. Specifically for *E. anchoita*, there are studies on dry and wet salting (ZUGARRAMURDI; LUPÍN, 1977). Studies on both acetic acid and salt uptake in fish are scarcer; Rodger et al. (1984) examined these diffusion properties in herring muscles.

In this work, the marinades of anchovies (*Engraulis anchoita*) were studied.

Engraulis anchoita is a small pelagic fish that occurs in the South Western Atlantic Ocean, from 22º S, in Brazilian waters, to 47º S, in Argentine waters. This species is the most abundant and underexploited pelagic fishes. The Argentinean process includes canned in oil, salted and matured product, salted fillet with oil and marinated. The last process mentioned is a new product

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developed with this species and there is scarce information with regard to this this process.

The global biomass value is 3.000.000 tons (SECRETARIA, 2008) and the Maximum Sustainable Yield is around 124,000 tons (NUESTRO, 2008) and at present it is underexploited at 28.197.9 tons (SAGPyA, 2007). With this statistical data it is possible to develop new products with this species. One alternative may be the elaboration of marinated anchovy. Thus, marinated Engraulis anchoita has been developed in Argentina. The procedure to make cold marinades from Engraulis anchoita was developed by Yeannes and Casales (1995) and then adopted by the industry using its own procedures and formulas. The effect of the process on the native flora was studied by Fuselli et al. (1994), the typical microbiological flora was isolated and characterized by Fuselli et al. (1998) and typical microorganisms in marinated anchovy fillets with corn oil and spices were determined by Fuselli et al. (2003). Physical and chemical changes in anchovy fillets during the marinating process were studied by Cabrer et al. (2002).

The cold marinating process consists of different stages: thawing, washing, heading, gutting and filleting, washing, brining and marinating. To design the marinating process, it is necessary to know the most important diffusional changes that occur in the food when it is immersed in a solution of low pH and high salt content.

The objective of this work was to study the simultaneous diffusion of acetic acid and sodium chloride in Atlantic anchovy (*Engraulis anchoita)* during marinating, to obtain equilibrium times and diffusion coefficients of acid and salt in order to design the marinating process.

2 Materials and methods

2.1 Marinating process

Two blocks of anchovies (*Engraulis anchoita*), each weighing 2 kg, caught in September, frozen on board and stored for two months at –30 °C, were used for the study.

The anchovies received the same treatment before marinating to obtain the diffusional results used during experimentation. The process (Figure 1) consisted of the following stages: thawing, washing, heading and gutting, filleting and cutting off the tail, washing, and brining in a brine bath with 10% of salt (food grade) for one hour, fish:solution ratio of 1:1 at room temperature (18 °C).

Anchovy fillets with the following chemical composition (expressed in % w/w): water 76.82, lipids 4.6, proteins 14.64, ashes 3.94 and sodium chloride 3.36 and pH 6.5 were immersed in the marinating solution. These fillets were 9.69 ± 0.29 cm long, 1.88 ± 0.07 cm wide and 0.61 ± 0.02 cm thick. These values represent the average of all measurements taken.

Marinating was carried out using the solution: fish ratio of 10: 1 to minimize concentration changes in the external solution. The marinating solution was composed of 3% v/v of acetic acid and 10% w/v of sodium chloride with a pH = 2,5.

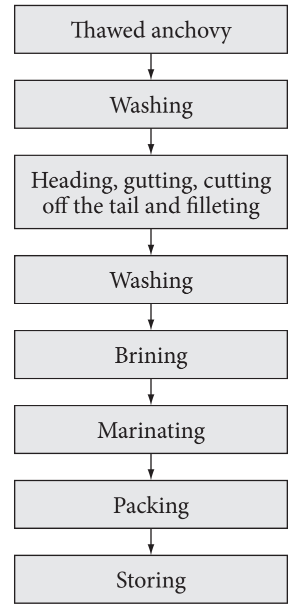

Figure 1. Marinating process of *Engraulis anchoita*.

Marinating was carried out in closed recipients at 20 ± 1 °C until equilibrium of salt and acetic acid between the anchovy fillets and the the marinating solution was reached. This stage was carried out with agitation (50 rpm) and without agitation. The agitation condition was incorporated to prevent the possible formation of a diluted surface layer on the fish fillets and to improve the diffusion of the solutes.

Two experimental runs were carried out. Determinations of water, sodium chloride and acetic acid contents and of pH were carried out in the anchovy fillets and in the marinating solution at the start of the process and at 0.5 hours intervals.

Physical and chemical analyses

Water content was determined at 105 °C until constant weight (AOAC., 1990). The sodium chloride content was determined as chloride using the Mohr method adapted to foods (KIRK et al., 1996). Acid content was determined by titration with sodium hydroxide (KIRK et al., 1996). The pH value was obtained with a pH meter (Instrumental Parsec, Vega VI) in a fish: distilled water ratio 1: 1 (AOAC, 1990).

All these determinations were carried out in triplicate, and water content for sextuple.

Water holding capacity (WHC)

The effect of salt and acid on WHC during the marinating stage was calculated as the variation between water content at different times (m_{water}) and the initial water content (m_{water0}) referred to the late value (GRAIVER et al., 2006) (Equation 1).

$$WHC = (m_{water} - m_{water0}) / m_{water0} \quad (1)$$

Distribution coefficients

The distribution coefficients defined as follows were calculated during marinating:

$$K(\text{salt}) = \frac{[\text{moles of sodium chloride / mass of water}]\ (\text{in the fillet})}{[\text{moles of sodium chloride / mass of water}]\ (\text{in the solution})} \quad (2)$$

$$K(\text{acid}) = \frac{[\text{moles of acetic acid / mass of water}]\ (\text{in the fillet})}{[\text{moles of acetic acid / mass of water}]\ (\text{in the solution})} \quad (3)$$

3 Results and discussion

According to Del Valle and Nickerson (1967a), Zugarrramurdi and Lupín (1976) studies on fish salting have shown that the equilibrium condition between fish muscle and brine was given by the equality of salt content in the brine and in the muscle tissue water Favetto et al. (1981) found that the equilibrium condition between beef and the external solution was given by the equality of sodium chloride and glycerol in the water in the solution and in the water in the muscle tissue, with small deviations within the limits of experimental error. In accordance with the aforementioned, the equilibrium of salt and acetic acid between the anchovy fillets and the marinating solution may be reached when salt and acid concentrations in the solution and in the fish muscle are equal.

The K values were obtained using Equations (2) and (3) at the time when equilibrium was reached. The equilibrium time was lower for samples without agitation during the marinating process (Table 1). The equality condition was reached in accordance with the distribution coefficients calculated close to unity.

The deviations observed for Sodium chloride may be due to the following:

- Not all the water in the fish muscle acts as solvent, a small amount is bound to specific groups of proteins (FAVETTO et al., 1981); and
- Not all the sodium chloride is in the solution, some may be bound to proteins (FAVETTO et al., 1981).

The equilibrium times for acid and salt were higher for the samples stirred during marinating.

At the equilibrium time the acetic acid and sodium chloride contents in the muscle were 1.75 and 5.42%, respectively, in samples without agitation and 1.71 and 5.56%, respectively, in samples with agitation, and the pH was 4.1 for samples with and without agitation.

Table 1. K values and Equilibrium times.

Marinating stage	K_{acid}	$T_{equilibrium}$ acid (hours)	K_{salt}	$t_{equilibrium}$ salt (hours)
Without agitation	1.01	2.80	1.00	2.20
With agitation	1.00	3.00	0.98	4.50

During marinating, the anchovy fillets lose water and gain acetic acid and salt. The net uptake of salt and acid expressed in a constant basis (fish solids) was used.

Figure 2 shows the values of WHC during marinating with and without agitation. The water holding capacity decreased, showing water loss during the analysis. WHC values for marinating with and without agitation were not significantly different ($p > 0.05$) for the first 3 hours and were significantly different ($p < 0.05$) for higher times. According to Rodger et al. (1984), in the presence of acid only, the pH of the muscle is on the acidic side of the isoelectric point and the electrostatic repulsion caused an open structure which allowed increased water holding, as salt is added these repulsive charges are shielded from one another and the structure then tightens up, causing decreased water holding.

The kinetics of sodium chloride uptake during the fish salting process was studied by Del Valle and Nickerson (1967b), Zugarramurdi and Lupín (1977, 1980). Studies on diffusion of salt and acetic acid in herring were performed by Rodger et al. (1984), the aforementioned authors demonstrated that sodium chloride and acetic acid uptake can be explained by Fick's law of non-steady state diffusion.

The diffusion of sodium chloride and acetic acid through the anchovy fillets can be considered as diffusion through a plane sheet of thickness 2l. At time $t = 0$, the region $-l < x < l$ is at uniform concentration (C_0) and the surfaces are kept at a constant concentration (C_1). The diffusion equation for one-dimensional motion can be expressed as follows (BIRD et al., 1964; CRANK, 1975; TURHAN; KALETUNC, 1992) (Equation 4):

$$\partial C/\partial t = \partial(D\partial C/\partial x)/\partial x \quad (4)$$

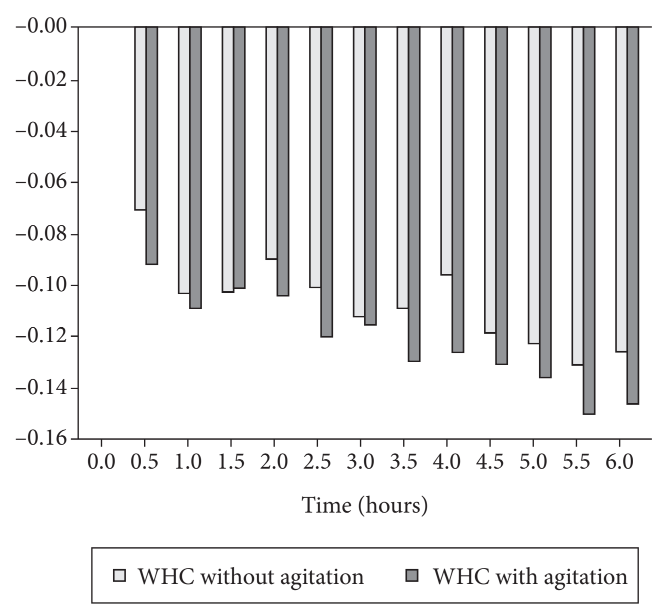

Figure 2. Water holding capacity during marinating stage.

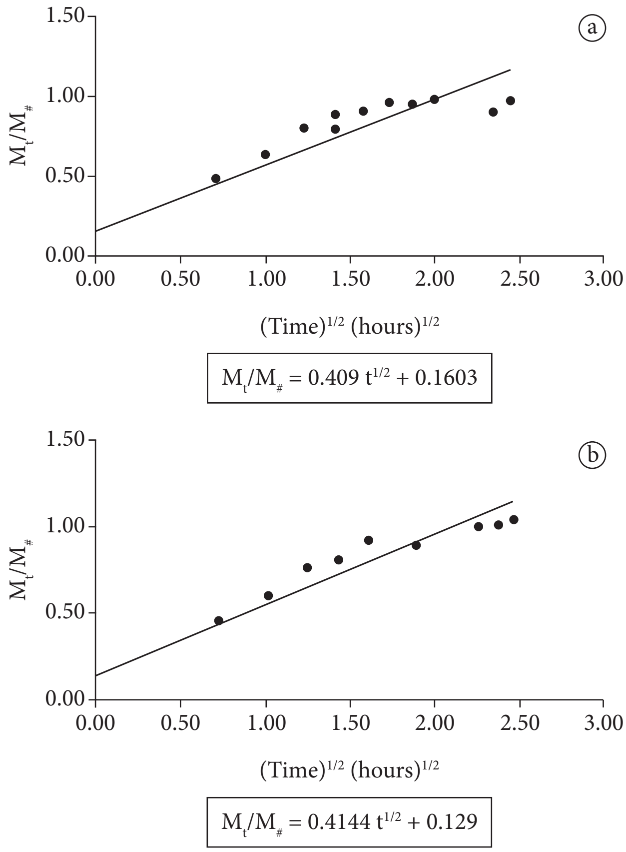

Figure 3. Diffusion of acetic acid during the marinating stage. a) Marinating with agitation; and b) marinating without agitation.

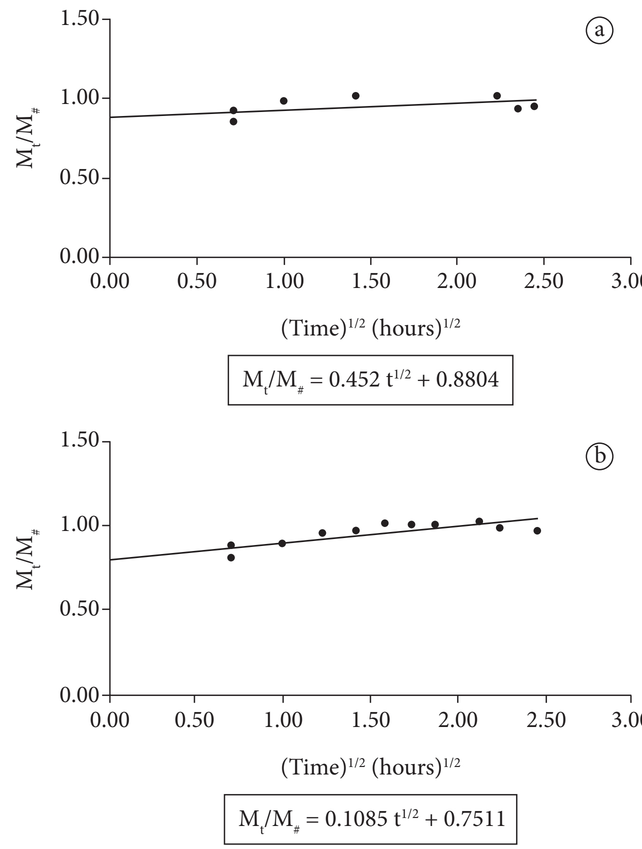

Figure 4. Diffusion of sodium chloride during the marinating stage. a) Marinating with agitation; and b) marinating without agitation.

where C is the concentration at time t and coordinate x, and D is the diffusion coefficient. If the diffusion coefficient is constant the equation can be expressed as follows (Equation 5):

$$\partial C/\partial t = D\, \partial^2 C/\partial x \quad (5)$$

The solution of Equation (3) is (Equation 6)

$$M_t/M_\infty = 2\,(Dt/\pi l^2)^{1/2} \quad (6)$$

where M_t is the total amount of diffusing solute which has entered the fillet at time t and M_∞ is the corresponding quantity after infinite time, l is ½ of thickness.

Since anchovy scales were eliminated and the influence of skin on the diffusion of salt and acid can be considered negligible for this species, half of thickness is used.

Figure 3 shows M_t/M_∞ against $t^{1/2}$, as seen for the early stages of sorption, M_t of acetic acid is proportional to $t^{1/2}$. The correlation coefficient (r) obtained was 0.928 ($p < 0.001$) and 0.956 ($p < 0.001$) for marinating with and without agitation, respectively (Figure 3a, b). The values of the diffusion coefficient for simultaneous diffusion of salt and acid at 20 °C may be obtained from the straight lines. The D value for acetic acid was 3.39×10^{-6} cm^2/seconds and 3.49×10^{6} cm^2/seconds for marinating with and without agitation, respectively. There was no difference between the diffusion coefficients.

Figure 4b shows M_t/M_∞ against $t^{1/2}$, as seen for the early stages of sorption M_t of sodium chloride is proportional to $t^{½}$ for marinating without agitation. The correlation coefficient (r) obtained was 0.898 ($p < 0,001$). The sodium chloride correlation coefficient was lower than the coefficients of acetic acid, this can be due to the effect of the brining stage that promotes an important uptake of sodium chloride arising at 3.36 w/w and decreasing the impulsive force. The value of D for sodium chloride was 2.39×10^{-7} cm^2/seconds for marinating without agitation. For marinating with agitation, there is a poor correlation between M_t/M_∞ and $t^{1/2}$ (Figure 4a) according to the r of 0.652 ($p < 0.01$). In this case, the D value could not be obtained.

Rodger et al. (1984) obtained diffusion coefficients for simultaneous diffusion of acid and salt in marinated herring. The D value for acetic acid for marinated herring is the same as the one obtained for marinated anchovy in this work. The diffusion coefficient for salt for marinated anchovy is lower than that obtained for herring. This can be due to the previous brining stage that increased the salt content of the anchovy fillet during marinating.

4 Conclusions

Acetic acid uptake in anchovy fillets during marinating with or without agitation can be explained by Fick's law. This is not the case for salt uptake. Correlation is also not found when marinating was performed with agitation. On the other hand, there is a good correlation when marinating was performed without agitation.

Agitation during marinating does not improve the uptake of sodium chloride and acetic acid and the equilibrium times were higher in samples stirred during marinating.

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